American Journal of Hematology 55:35–38 (1997)

Thirty-One-Year Survival Following Chemotherapy and Autologous Bone Marrow in Malignant Lymphoma

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A 21-year-old woman with malignant lymphoma received a large dose of nitrogen mustard followed by autologous bone marrow. She achieved a complete remission that lasted 21 years. This was followed in a span of 10 years by two relapses that responded to chemotherapy and splenectomy. Death resulted from sepsis. At autopsy, there was minimal evidence of malignant lymphoma, but evidence for side effects of chemotherapy was present. Am. J. Hematol. 55:35–38, 1997. © 1997 Wiley-Liss, Inc.

Key words: autologous bone marrow; chemotherapy; malignant lymphoma

INTRODUCTION

During the 1950s, the late Leandro M. Tocantins, M.D., then the Director of the Cardeza Foundation for Hematologic Research of Jefferson Medical College (now part of Thomas Jefferson University), was among the primary movers of human bone marrow transplantation [1–4]. As his associate, we treated 26 patients with bone marrow transplantation, mainly following total body radiation or chemotherapy for various bone marrow-depleted syndromes [1]. The latter consisted mainly of acute leukemia, malignant lymphoma, and aplastic anemia. In general the results were not satisfactory and remained so until Dausset and others established the HLA system of tissue histocompatibility [5]. However, one of our patients who had a malignant lymphoma achieved an excellent result. The patient, a 21-year-old college student, was treated with intensive chemotherapy and autologous bone marrow transplantation. She was among the first to receive such a procedure, and I believe the first so far to have an excellent result lasting 31 years [1]. Her story is the basis of this report.

CASE REPORT

The patient, M.G. was a 21-year-old woman from Harrisburg, Pennsylvania, who was a junior student at Duke University. She was admitted to Jefferson Hospital in July 1959. Her symptoms were mainly fatigue and 10 pounds of weight loss of 5 months' duration and bilateral swelling of the neck of 4 months' duration. Physical examination revealed a young patient who did not look overtly sick. There was a low-grade fever and generalized peripheral lymphadenopathy. The lymph nodes, of variable size, were discrete, movable, firm, and nontender. The spleen was palpable 2 cm below the coastal margin on deep inspiration. Chest film revealed hilar and paratracheal lymphadenopathy.

The peripheral blood picture revealed a hemoglobin (Hb) of 11.0 g/dl, red blood cell (RBC) count 3.7 million/mm^3, a reticulocyte count of 2.6%, a white blood cell (WBC) count of 5,700/mm^3 with slight increase in lymphocytes and monocytes. Platelet count was 375,000/mm^3. The bone marrow was essentially normal. Blood chemistry was normal, except for slight changes in albumin and γ-globulin. An inguinal lymph node biopsy was diagnosed as reticulum cell sarcoma arising from follicular lymphoma. The diagnosis was confirmed by Dr. Philip Custer, at the time a leading authority on hematopathology. The same biopsy slides were reviewed a decade later by several nationally leading hematopathologists, and the diagnosis was updated to nodular, mixed (lymphocytic and histiocytic), poorly differentiated malignant lymphoma.

It was decided, as part of the treatment, to aspirate the patient's marrow before administration of chemotherapy. Under general anesthesia, 350 ml of the patient's bone marrow was aspirated from multiple sites; sternum, iliac

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Received for publication 18 May 1996; Accepted 14 November 1996.

crests, and iliac spines. The marrow was delivered to a phosphate-buffered sequestrene solution. Glycerol was added to give a final concentration of 15%. The marrow was frozen slowly and stored at −65°C. Four days later, patient whose weight was 55 kg received 25 mg of nitrogen mustard IV every other day, for a total dose of 100 mg (1.8 mg/kg). On the last day of chemotherapy, the bone marrow was completely aplastic. Two days later, the patient received her autologous marrow intravenously after it was thawed. She received 4.9×10^9 nucleated cells intravenously. Stained smear of the material showed intact nucleated bone marrow cells of those counted. During the next 36 hr, she passed 13.6 g of Hb in the urine from her hemolyzed glycerated red cells.

The patient developed the expected complications; fever, mouth ulcers, nausea, and loss of hair. She received supportive therapy in the form of antibiotics, blood transfusions (some were direct from donors to provide fresh and viable platelets), antinausea medication, and sedation. As chemotherapy was administered, the patient developed severe cytopenia, which lasted 15 days from the day the autologous marrow was administered. Recovery was heralded by an increase in the 24-hr urinary excretion of uric acid on the twelfth day from the marrow infusion. Before the procedure, the urinary daily output of uric acid was three times normal, after which it dropped to almost zero during the aplastic phase. The fever disappeared 5 days before the rise in the formed elements of the blood. Granulocytes, platelets, and reticulocytes recovered in that order, a few days within each other. The granulocytes reached the 500 count on the 15th day post-transplantation and doubled the following day. The platelet count was more than 20,000 on the 17th day. All formed elements of the blood became normal or above normal on the 20th day post-autologous bone marrow transfusion. The lymphadenopathy and splenomegaly disappeared. The patient fully recovered and was discharged after a 2-month stay in the hospital. Two bone marrow aspirations before discharge were hypercellular but normal.

The patient returned for a follow-up visit 6 months later. She was in a complete remission. She continued her education and received a bachelor's degree in French from Duke University in 1961 and a master's degree the next year from the University of Florida. She got married, moved to Tallahassee, Florida, and had three children. M.G.P. became an astute artist; calligrapher (studied in Wales under the Queen's calligrapher), watercolor painter (studied at London Royal College of Art), and pianist. At one time she was president of the LeMoyne Art Foundation and served on the City Hall Art Council. During all these activities, she continued to have regular medical checkups and continued in complete remission for 21 years.

In May 1980, during a routine checkup, M.G.P. was found to have a recurrence of peripheral lymphadenopathy. A hemogram revealed a Hb of 12.6 g/dl, hematocrit (Hct) of 36%, RBC count of 3.8 mil./mm^3, platelet count of 314,000/mm^3, and a WBC count of 16,500/mm^3. Neutrophils 25% and lymphocytes 75% with a chromatin pattern that appeared mature, but some were cleaved. A bone marrow biopsy from the left posterior iliac crest revealed large lymphoid nodules which centrals were composed of poorly differentiated mixed lymphocytic and histiocytic cells. Diffuse areas of lymphoma appeared pressed against bone trabeculae. There were relatively normal areas of bone marrow with megakaryocyte, erythroid, and myeloid precursors. Bilateral inguinal lymph node biopsies revealed a similar pattern, a mixed lymphocytic and histiocytic nodular type. The histiocytic component was slight, and the predominant cell type was that of poorly differentiated cleaved lymphocyte. There were occasional mitotic figures.

The patient received a course of chemotherapy, consisting of vincristine, cytoxan, procarbazine, and prednisone for 2 weeks every month for 6 months. Patient entered a complete clinical remission. At the completion of chemotherapy, her bone marrow revealed no malignant lymphoma. She did well until she relapsed in April 1989. She felt tired, and the lymphadenopathy recurred. A second course of chemotherapy was administered, consisting of vinblastine, cytoxan, and prednisone. Because of a huge symptomatic spleen, the patient underwent splenectomy in August 1990. The spleen weighed 1,580 g. There was effacement of the normal architecture with areas of infarction and necrosis, but the predominant feature was the nodular mixed (histiocytic and poorly differentiated cleaved lymphocytes) malignant lymphoma. Another course of chemotherapy was instituted.

Three days after receiving an injection of chemotherapy, the patient collapsed at home and died on September 6, 1990. Death was presumed to be secondary to septic shock. Autopsy revealed sparse residual lymphomatous elements in a hypocellular bone marrow; the myeloid and erythroid elements were markedly diminished; mild to moderate generalized lymphadenopathy (edema, fibrosis, congestion with marked diminution of lymphocytic component), acute segmental ischemic colitis and diverticulitis, multiple gastric ulcers and lower esophageal ulceration (probably *Candida* invasive growth), and pulmonary edema with bacterial colonization. Liver, lungs, and kidneys showed edema and congestion with bacterial colonization but no lymphoma. Blood culture revealed mixed growth of *Proteus, Streptococcus,* and *Klebsiella.*

DISCUSSION

M.G.P. was an unusual patient. She was the first to receive an autologous bone marrow transplantation and

to live more than 30 years, at least two-thirds of which was in a state of complete remission. During those years, she led a full and active life. The malignant lymphoma, though of an aggressive type, was sensitive to chemotherapy until the very end. At autopsy, limited disease was present, confined primarily to the lymph nodes and none in internal organs. However, by then, the bone marrow reserves were exhausted, and the patient succumbed from the usual side effects of chemotherapy, which had served her well for 30 years.

During the first relapse, she received nitrogen mustard, a by-product of the war efforts of World War II. At that time, in 1959, it was the treatment of choice for malignant lymphoma. She received a whopping dose, which no doubt eradicated the lymphoma, since she lived for 21 years in complete remission. It is of interest that nitrogen mustard, no longer in use as a therapeutic agent for malignant lymphoma, was very helpful to the patient. It controlled the disease for many years and had no delayed side effects, whether related to the patient, her fertility, or progeny. Perhaps it should be reconsidered as a chemotherapeutic agent for malignant lymphoma.

Whether the relapse was due to some lingering malignant cells that survived the chemotherapy or was reintroduced with the autologous transplant (at the time, no purging procedures for bone marrow was available) cannot be ruled out. However, more likely, the factors responsible for the development of the malignant lymphoma in the first place recurred and led to the relapse.

Autologous bone marrow transplantation helped this patient recover sooner, if it did not save her from a worse outcome. The injected cells helped tide her over the aplastic phase. What was responsible for repopulation of the bone marrow? Was it the autologous bone marrow or the few stem cells that might have survived the onslaught of chemotherapy and lurked behind? The data submitted here are more supportive of the first suggestion, but the last cannot be excluded. It took the formed elements of the peripheral blood 2 weeks to start to come back. If the marrow in situ was responsible for repopulation after an overwhelming dose of nitrogen mustard, one would expect a longer period of aplasia, of 3 weeks or more. Also, the subsiding fever and the rising 24-hr urinary uric acid indicated a bone marrow recovery on the twelfth day after the administration of the autologous bone marrow. Subsiding fever and rising 24-hr urinary uric acid were good early indicators of bone marrow recovery [1]. Urinary uric acid is a combination of dietary factors and DNA turnover. In this case, the patient was receiving intravenous fluids and no meals, except for high protein and carbohydrate liquids during the aplastic phase, so the urinary uric acid output reflected mainly the DNA turnover of the bone marrow.

Currently, bone marrow transplantation is almost a conventional mode of therapy. Earlier, it was an experimental approach. The bone marrow transplantation experiment arose as an outcome of World War II and the atomic age heralded by the Manhattan Project and the bombing of Hiroshima and Nagasaki. The U.S. Atomic Energy Commission provided us and others with money for research to prevent, contain accidents, and/or treat casualties if an accident occurred. Potential accidents could arise from atomic submarines harbored at Seattle or under the oceans elsewhere. They could also arise from atomic reactors during peace time and from atomic wars.

The data collected at Hiroshima and Nagasaki following the atomic bombs provided information on the human effects of radiation. For those who did not die immediately from radiation, hematologic complications developed, of which leukemia was the outstanding [6]. The immediate radiation injuries and deaths were central nervous system deaths after exposure to 7,500 rads; gastrointestinal deaths within days upon receiving a lesser dose of 1,500 rads; and bone marrow failure deaths within weeks following exposure to a dose of 800 rads. The following definitions were coined: *sublethal,* injury but no deaths; *midlethal,* some deaths; and *supralethal,* 100% deaths. Animal experimentation started in order to reverse some of the ill effects of radiation. Anticonvulsive drugs, fluids, electrolytes, and antibiotics were found to improve survival in dogs exposed to various doses of radiation. The LD_{50} was defined as the radiation dose calculated to give 50% survival at 30 days [7].

The bone marrow transplantation experiment started with Jacobson et al. [8] at the University of Chicago, also home of the Manhattan Project. These investigators shielded the exteriorized spleen of various animals in parabiosis before exposing them to total body radiation. The recovery of the animals was explained by the presence of repopulating cells, humoral factors, and/or a radiation detoxification process in the spleen. The definitive bone marrow transplantation experiment was performed by Lorenz et al. [9] at the NIH. The group showed that lethally radiated (X-ray, γ, or β, but much less against neutrons) animals were kept alive after isologous bone marrow transplantation. The LD_{50} of 600 rads could be doubled with isologous 10×10^6 cells. The relationship was established for the number of marrow cells needed to recovery from a lethal dose of radiation for different types of marrow cells: autologous (self), homologous (same strain), isologous (same species), and heterologous (different species). The end result of bone marrow transplantation is a ''take.'' If that happens, graft-versus-host reaction could follow if isologous bone marrow cells were used.

The original work by the British team led by Barnes and Loutit [10,11] on murine leukemia led, or at least encouraged, experimentation on human bone marrow transplantation for the treatment of acute leukemia and

other bone marrow-depleted syndromes. These investigators showed the beneficial effect of total body radiation followed by normal homologous bone marrow transplantation on murine leukemia. More or less at the same time that Haurani, Repplinger, and Tocantins were carrying on their human bone marrow transplantation trials [1–4], other groups were also performing this procedure, primarily in acute leukemia, or were advocating the procedure: Ferrebee and Thomas in Cooperstown, N.Y. [12], Dameshek's group in Boston [13], Georges Mathe in France [14], and van Bekkum in Holland [15].

On the whole, the results of these attempts at human bone marrow transplantation in bone marrow depleted syndromes were not encouraging. Human bone marrow transplantation took a back burner position for many years. It was the discovery of human tissue typing (HLA system) that brought back the bone marrow experiment to the front burner, and the results improved significantly. Still today, the bugaboo of bone marrow transplantation is graft-versus-host reaction. A few advances have been made in this area with immunosuppressive therapy, including the use of methotrexate and cyclosporine.

The story of histocompatability began with Peter Medawar in 1949 [15]. He observed that skin grafts were rejected if the animals previously received an intradermal injection of homologous leukocytes. Gorer is the father of the histocompatability antigens. He identified in a murine tumor system antigens that were common with leukocytes [16]. The systemic study of human leukocyte antigens began in the mid-1950s by Jean Dausset in France [17]. The results of these human experimentation studies, including skin grafts performed by Dausset and Felix Rapaport, and the basic studies conducted by van Rood and Eermisse [18], among others, put tissue typing on the map and are responsible for making human bone marrow transplantation conventional therapy today.

ACKNOWLEDGMENTS

The author thanks Julius Frederick Parker, Jr., Esq. (husband), Drs. J. Brian Sheedy and Thomas P. Wood (Tallahassee, FL), for valuable information about the deceased. Also the author thanks Yolanda Capo (Department of Hematology of the University of Miami Medical School) and Nina Zimmer (Medical record Department, Thomas Jefferson University Hospital, Philadelphia) for helping in tracing the deceased, as well as old medical records. Without the support and cooperation of these people, this report would not have been possible.

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